Thermal destruction of *Listeria monocytogenes* in liver sausage slurry

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Thermal destruction of Listeria monocytogenes *was determined in a liver sausage slurry (1:1, liver sausage batter and water) using a submerged ampule technique.* D-*values for* L. monocytogenes *Scott A grown at 37°C were 8·9 min at 57·2°C, 2·4 min at 60·0°C, and 1·1 min at 62·8°C (Z=6·2°C) based on analysis of the linear portion of the survivor curves.* D-*values of 6·6, 1·6, and 0·4 min (Z=4·65°C) were obtained when the data were analyzed using non-linear techniques.* L. monocytogenes *strain V7 (D_{60}=1·0 min) was more thermosensitive than Scott A (D_{60}=1·6 min) or HO-VJ-S (D_{60}=1·6 min). When Scott A was grown at 19°C, there was a decrease in thermal resistance (D_{60}=0·8 min). These data indicate that* L. monocytogenes *has a thermal resistance in liver sausage comparable to that observed in other food systems.*

Introduction

There have been a number of detailed studies of the thermal resistance characteristics of *Listeria monocytogenes* during pasteurization of dairy products (Mackey and Bratchell 1989, Knabel et al. 1990, Mackey et al. 1990, Pearson and Marth 1990); however, substantially less information is available concerning the fate of the pathogen during thermal processing of meat products (Mackey and Bratchell 1989, Boyle et al. 1990, Johnson et al. 1990, Mackey et al. 1990). Zaika et al. (1990) investigated the destruction of *L. monocytogenes* during the smoke-house processing of frankfurters, determining that heating to a core temperature of 71·1°C (160°F) gave an approximate 1000-fold reduction in viable counts. As an integral part of a study to determine the appropriate conditions for elimination of listeriae during liver sausage processing, we report on the thermal resistance of *L. monocytogenes* in a liver sausage slurry as determined using a submerged ampule technique.

Materials and Methods

Micro-organism and growth conditions

Three strains of *L. monocytogenes* were used for these studies: Scott A (clinical, FDA), V7 (raw milk, FDA), and HO-VJ-S (raw ground beef, USDA). Unless otherwise indicated, storage of cultures, preparation of inocula, and incubation conditions were as described previously by Zaika et al. (1990). Inocula were prepared by growing the organism in 100 ml Brain Heart Infusion Broth (Difco, Detroit, MI) with 0·3% added glucose for 18 h with shaking at 200 rpm at either 37 or

19°C. The culture was centrifuged at 10 400 *g* (5°C, 10 min), the cell pellet resuspended in 5 ml of sterile distilled water and the entire suspension was used as the inoculum.

Preparation of liver sausage slurry

Liver sausage slurry was prepared by adding 100 g frozen pork liver, 100 g frozen pork trimmings, 2·09 g commercial spice mixture, 4 g sucrose, 10 g sodium chloride, and 62·4 mg sodium nitrate with 200 ml of sterile water in a sterile blender jar. The slurry was prepared by mixing all ingredients for 30 s at high speed in the blender jar; then, the resuspended cells were added and the cell-slurry was further mixed at high speed for an additional 30 s. The pH of the liver sausage slurry was 6·5.

Thermal inactivation and enumeration

The heat resistance of each strain was tested separately by inoculating the slurry with individual strains of *L. monocytogenes* to give an initial count of 1×10^9 cfu ml^{-1}. The slurry was then transferred in 3·5 g portions to sterile 60×17-mm screw neck vials. The vials were sealed with a sterile rubber septa and lid and submerged in a constant temperature water bath held at 57·2, 60·0, or 62·8°C. Internal temperature was monitored continuously by a thermocouple (Type T copper-constantan, Baily Instruments, Saddle Brook, NJ; the thermocouple joints were welded in our shop). The temperature was recorded using model MRL250 multipoint recorder logger (Esterline Angus Instrument Corporation, Indianapolis, IN) inserted at the center of an uninoculated vial. The times required for the inoculated liver sausage slurry to reach the internal temperature of 57·2°C, 60·0°C, and 62·8°C were 3·50, 3·75, and 4·00 min, respectively. Processing times were calculated from the time when the internal temperature of the slurry reached the target level. At intervals, vials were removed and cooled in crushed ice. The time for the internal temperature of the vial to cool to ≤35°C was 30 s, indicating that there was little, if any, thermal destruction after vials were removed from the heating bath.

Samples were processed by transferring a 1·1 g portion of slurry to a sterile 6 oz Whirl-Pak™ bag containing 9·9 ml of 0·1% sterile peptone (Difco) water. Each bag was processed for 2 min in a Stomacher Lab-blender (Model 400, Spiral Systems, Inc). Serial dilutions were made in 0·1% peptone water and surface plated on duplicate tryptose agar (Difco) plates. The plates were incubated at 37°C for 72 h and typical *Listeria* colonies were counted. Colonial identity (3–5 colonies) was verified by standard biochemical techniques as described by Zaika et al. (1990).

D-values were calculated by regression analysis of the linear portion of the survivor curves. Additionally, the sigmoidal character of the observed survivor curves prompted reanalysis using the Gompertz equation (Gibson et al. 1987) as described by Buchanan et al. (1989). In both instances, the *D*-value was the negative reciprocal of the slope. *Z*-values were calculated from duplicate experiments by linear regression of the *D*-values.

Results and Discussion

The fate of *L. monocytogenes* Scott A heated to temperatures of 57·2°, 60·0°, and 62·8°C in liver sausage slurry is illustrated in Fig. 1. Virtually no killing occurred during the come-up period (data not presented). Both a shoulder and tailing were observed at all temperatures. Analysis of the curves using the Gompertz equation allowed quantitation of the duration of the shoulder. This lag period for cells heated at 57·2°, 60·0°, and 62·8°C was 23·0, 9·2, and 3·0 min respectively, indicating an inverse relationship between heating temperature and the time to initiate thermal destruction. Mackey et al. (1990) also observed shoulders during the thermal processing of meat, particularly when fat and curing salts were added to the meat mixture. They attributed this extension of lag period to the curing salts, which were similar to those used in the liver slurry.

D-values calculated by linear and non-linear regression methods are presented in Table 1. The values obtained by the two methods were similar. However, since the calculation of *D*-values using the Gompertz equation takes advantage of all the data points, it is likely to provide a more accurate estimate of an micro-organism's thermal resistance

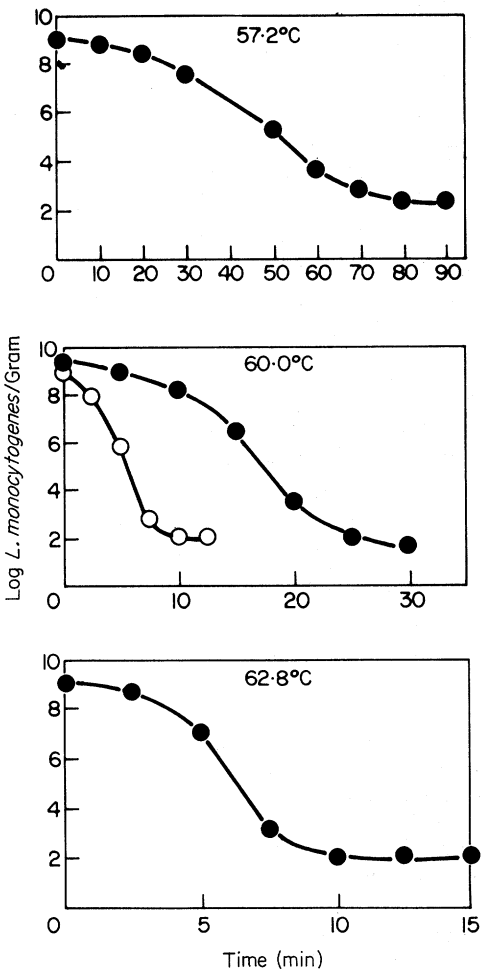

Fig. 1. Thermal destruction of *L. monocytogenes* Scott A at 57·2, 60·0, and 62·8°C in liver sausage slurry (1:1). The cells were grown at 37°C (•—•) or at 19°C (o—o) prior to heat treatment.

when dealing with sigmoidal survivor curves. *Z*-values derived from the two sets of *D*-values were similar (Table 1). Other workers utilizing strain Scott A reported D_{60} values of 2·5 min in a 1·4 ground beef and water slurry (Boyle et al. 1990), 8·3 min in a thick slurry of beef (Mackey and Bratchell 1989), and 5·3 min in thick slurry of chicken (Mackey and Bratchell 1989). Mackey and Bratchell (1989) reported further that the *Z*-value for *L. monocytogenes* Scott A was 6·0°C in both beef and chicken slurries.

Since *L. monocytogenes* can grow well at low temperatures, cells grown at 19°C were also tested to assess the impact of growth temperature on thermal resistance. The D_{60} for 19°C grown cells was 0·8 min as compared to 1·6 for 37°C grown cells (Fig. 1). It has been shown that microbial cells grown at low temperatures are less thermotolerant (Hansen and Rieman 1963, Beuchat 1978); however, these studies did not utilize food systems. Recent work by Smith et al. (1990) indicated that *L. monocytogenes* grown at even lower temperatures would likely be even less heat resistant.

Thermal destruction of *L. monocytogenes* in liver sausage slurry was assessed further by examining the thermal resistance of strains V7 and HO-VJ-S. The *D*-values at 60°C were 1·0 min and 1·6 min, respectively. Thus, strain V7 was less thermotolerant than Scott A

Table 1. Thermal destruction of Scott A strain in liver sausage slurry

Temperature (°C)	*D*-value (min)[a]		*D*-value (min)[b]	
	Mean	s.d.	Mean	s.d.
57·2	8·91	0·02	6·57	0·30
60·0	2·42	0·12	1·57	0·07
62·8	1·12	0·06	0·43	0·05
Z	6·20°C	0·10	4·65°C	0·15

D-values were calculated as described in Materials and Methods, and are the means of duplicate determinations.
[a] Calculated from linear portion of thermal death time curve.
[b] Calculated by using Gompertz equation.
s.d., Standard deviation.

(D_{60}=1·6 min), whereas HO-VJ-S had similar thermal resistance. In other food systems, such as dairy products, Scott A was found to be the most heat resistant strain (Pearson and Marth 1990).

To the best of our knowledge, the current study represents the first application of the Gompertz equation to the analysis of inactivation kinetics data. While the traditional method for obtaining *D*-values for data sets which show only a linear response between log (survivor counts) and heating time is adequate, the Gompertz equation appears to have distinct advantages when dealing with non-linear survivor curves which have an initial shoulder and tailing, including allowing use of all data points and providing a means for quantifying the duration of the lag period. This type of survivor curve is observed commonly when studying inactivation kinetics of vegetative cells at low thermal processing temperatures such as those used for cooking meat products.

Liver sausage is normally processed so that it reaches an internal temperature of 65·6–66·7°C (150–152°F) (Kramlich et al. 1973). Based on our data for thermal inactivation of Scott A, the most thermoresistant strain studied (D_{60}=1·6 min, Z=4·65°C), holding liver sausage for 1–2 min at 65·6°C should ensure the complete destruction of any listeriae present. However, this conclusion is confounded by the observation of significant tailing, and assumes a linear extrapolation of the *Z*-value calculation plot. Future experiments are underway to test this supposition utilizing liver sausage processed in a smoke house.

References

Beuchat, L. R. (1978) Injury and repair of gram-negative bacteria, with special consideration of the involvement of the cytoplasmic membrane. *Adv. Appl. Microbiol.* **23**, 219–243.

Boyle, D. L., Sofos, J. N. and Schmidt, G. R. (1990) Thermal destruction of *Listeria monocytogenes* in a meat slurry and ground beef. *J. Food Sci.* **55**, 327–328.

Buchanan, R. L., Stahl, H. G. and Whiting, R. C. (1989) Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrate on the growth of *Listeria monocytogenes*. *J. Food Protect.* **52**, 844–851.

Gibson, A. M., Bratchell, N. and Roberts, T. A. (1987) The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridum botulinum* type A in pasteurized pork slurry. *J. Appl. Bacteriol.* **62**, 479–490.

Hansen, N. H. and Rieman, H. (1963) Factors affecting the heat resistance of nonsporing organisms. *J. Appl. Bacteriol.* **26**, 314–333.

Johnson, J. L., Doyle, M. P. and Cassens, R. G. (1990) *Listeria monocytogenes* and other Listeria spp. in meat and meat products: a review. *J. Food Protect.* **53**, 81–91.

Knabel, S. J., Walker, H. W., Hartman, P. A. and Mendonca, A. F. (1990) Effects of growth temperature and strictly anaerobic recovery on the survival of *Listeria monocytogenes* during pasteurization. *Appl. Environ. Microbiol.* **56**, 370–376.

Kramlich, W. E., Pearson, A. M. and Tauber, F. W. (1973) *Processed meats.* Westport, CT, AVI Publishing Co. Inc.

Mackey, B. M. and Bratchell, N. (1989) The heat resistance of *Listeria monocytogenes. Lett. Appl. Microbiol.* **9**, 89–94.

Mackey, B. M., Pritchel, C. and Mead, G. C. (1990) Heat resistance of *Listeria*: Strain differences and effects on meat type and curing salts. *Lett. Appl. Microbiol.* **10**, 251–255.

Pearson, L. J. and Marth, E. H. (1990) *Listeria monocytogenes*—threat to a safe food supply: a review. *J. Dairy Sci.* **73**, 912–928.

Smith, J. L., Marmer, B. and Benedict, R. C. (1990) Influence of growth temperature on injury and death in *Listeria monocytogenes.* In *Abst. Ann. Meet. Soc. Microbiol.*, p. 286. Anaheim, CA.

Zaika, L. L., Palumbo, S. A., Smith, J. L., Del Corral, F., Bhaduri, S., Jones, C. O. and Kim, A. H. (1990) Destruction of *Listeria monocytogenes* during frankfurter processing. *J. Food Protect.* **53**, 18–21.